Elevated Stress Hormone Levels Relate to Epstein-Barr Virus Reactivation in Astronauts

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Objective: The objective of this study was to determine the effects of stress and spaceflight on levels of neuroendocrine hormones and Epstein-Barr virus (EBV)–specific antibodies in astronauts. **Methods:** Antiviral antibody titers and stress hormones were measured in plasma samples collected from 28 astronauts at their annual medical exam (baseline), 10 days before launch (L−10), landing day (R+0), and 3 days after landing (R+3). Urinary stress hormones were also measured at L−10 and R+0. **Results:** Significant increases ($p < .01$) in EBV virus capsid antigen antibodies were found at all three time points (L−10, R+0, and R+3) as compared with baseline samples. Anti-EBV nuclear antigen antibodies were significantly decreased at L−10 ($p < .05$) and continued to decrease after spaceflight (R+0 and R+3, $p < .01$). No changes were found in antibodies to the nonlatent measles virus. The 11 astronauts who showed evidence of EBV reactivation had significant increases in urinary epinephrine and norepinephrine as compared with astronauts without EBV reactivation. **Conclusion:** These findings indicate that physical and psychological stresses associated with spaceflight resulted in decreased virus-specific T-cell immunity and reactivation of EBV. **Key words:** Epstein-Barr virus, stress, spaceflight, microgravity, viral immunity.

EBNA = Epstein-Barr virus nuclear antigen; EBV = Epstein-Barr virus; ELISPOT = enzyme-linked immunospot assay; IgG = immunoglobulin G; L−10 = launch minus 10 days; R+0 = return/landing day; R+3 = 3 days after landing; VCA = viral capsid antigen.

INTRODUCTION

A number of studies have shown that immune function in astronauts is decreased during and after spaceflight. Decreased interferon production, altered distribution of leukocyte and lymphocyte subsets, and decreased delayed-type hypersensitivity have been observed (1–6). It has been proposed that stress may be a major mediating factor in the impaired immune response of astronauts (1, 4, 7). Importantly, stress may result in decreased cellular immunity and predispose individuals to viral infections (8, 9).

To address viral infections in astronauts, we investigated a latent herpesvirus, specifically Epstein-Barr virus (EBV), and its potential as a marker of decreased immune function. EBV remains latent in healthy individuals and undergoes occasional reactivation after primary infection (10). Decreased cellular immunity against EBV permits productive cycles of viral replication, which lead to increased production of EBV lytic antibodies (11). Immunosuppressed individuals also have decreased or absent anti–EBV nuclear antigen (EBNA) antibodies (11–13), which is thought to reflect decreased or absent cytotoxic T-cell function against EBV (14). In support of this interpretation, a positive correlation between anti-EBNA antibody levels and precursor frequency of EBV-specific cytotoxic T-cells has been demonstrated (15). In our initial study, we found increased antibodies to EBV viral capsid antigen (VCA) before flight and 8- to 64-fold increases in EBV early antigen antibodies after spaceflight (16). These findings suggested that chronic stress occurred before flight and that in-flight events (possibly associated with the stress of launch and landing) triggered replication of EBV.

Urinary cortisol and catecholamines were also elevated after flight in this study (16). Elevated levels of cortisol have also been documented during spaceflight (17, 18). Importantly, glucocorticoids downregulate cellular immunity as well as directly reactivate EBV (19–24). The purpose of this study was to measure anti-EBNA antibodies to determine if virus-specific T-cell immunity was decreased. In addition, analyses of the stress hormone data in this study focus on differences between astronauts who showed evidence of EBV reactivation and those who did not.

MATERIALS AND METHODS

Subjects

Three separate samples of peripheral blood were collected from each of the 28 astronauts, all EBV seropositive, who flew aboard five US Space Shuttle flights. The 23 male and 5 female astronauts were between the ages of 36 and 59 years (mean, 42 ± 5 years). The Johnson Space Center Institutional Review Board approved this study, and informed consent was obtained from all subjects before participation.

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Design

Peripheral blood was collected in Vacutainer tubes coated with ethylenediaminetetraacetic acid 10 days before launch (L−10), within 4 hours of return/landing (R+0), and 3 days after landing (R+3). Preflight (L−10) and postflight (R+3) blood draws were performed early in the morning (around 7:30 AM) and processed at the Johnson Space Center. Blood collected immediately after spaceflight (R+0) took place at the Kennedy Space Center. Sample collection times at R+0, which varied with the landing time, usually were in the early afternoon. Plasma was obtained after centrifugation and was stored at −70°C until testing. Twenty-four hour urine samples, collected from each crew member at L−10 and R+0, were stored at −20°C until analysis. Baseline anti-viral antibody titer data, routinely measured only once during the astronaut's first annual medical exam (average of 22 months before flight), were obtained from archived records. Baseline values for anti-VCA antibodies were available for all astronauts; values for anti-measles virus antibodies and anti-EBNA antibodies were available for only 14 and 16 crew members, respectively. Peripheral blood was also drawn 15 days apart on 14 healthy subjects (6 men and 9 women, mean age = 33 ± 6 years) for comparison with the astronauts' results.

Measurement of Antiviral Antibody Titers

The measurement of anti-VCA IgG antibody titers and anti-measles virus IgG antibody titers has been described in detail (16). Commercially prepared substrate slides and control sera were used for determining antibody titers to EBNA IgG (Bion Enterprises, Park Ridge, IL). Serum samples were first heated at 56°C for 30 minutes to inactivate endogenous complement. Sera was then added to the spot slides, and the slides were incubated at 37°C for 30 minutes and then washed for 5 minutes in phosphate-buffered saline. Guinea pig complement (Colorado Serum Company, Denver, CO) was quickly thawed, diluted 1:10 in cold phosphate-buffered saline, and pipetted onto the spot slides. After a second incubation (37°C for 30 minutes), the slides were washed, and one drop of anti-guinea pig complement fluorescein conjugate was applied to each well of the slide. A final incubation of 37°C for 30 minutes was performed, and then the slides were washed and mounted. The slides were then examined and evaluated by fluorescence microscopy. Two-fold dilutions of plasma or serum from each subject were prepared, and the endpoint titer was determined as the highest dilution still able to demonstrate immunofluorescence-positive cells. All specimens were batch analyzed and read blind-coded.

Measurement of Stress Hormones

The measurement of hormones and immunoglobulins has been described previously in detail (8, 21). Plasma and urinary cortisol were measured by radioimmunoassay. Urinary catecholamines (epinephrine and norepinephrine) were measured by high-performance liquid chromatography. Samples were batch analyzed to minimize interassay variation.

Statistical Analysis

Normality was assessed using the Kolmogorov-Smirnov normality test. Data not normally distributed were subjected to natural log transformations to normalize the distributions before analyses. Because the method of doubling dilutions was used to obtained antibody titer results, a log base-2 transformation was used to reduce variance for statistical comparisons. The natural logarithms of plasma cortisol, urinary epinephrine, and norepinephrine passed the normality test and were used for paired *t* test statistical analysis. The nonparametric Wilcoxon signed-rank test was used for urinary cortisol, which did not pass even after transformation. Repeated-measures analysis of variance was used to analyze anti-EBNA IgG, anti-VCA IgG, and anti-measles IgG across multiple time points. The percentage of change in each hormone for each subject was calculated as follows: [(R+0 level − L−10 level)/L−10 level] × 100. Statistically significant differences between groups were assessed by the Fisher exact test. Results are expressed as mean ± SE, and $p < .05$ was considered significant.

RESULTS

A significant decrease ($p < .05$) in the anti-EBNA IgG was found at L−10 as compared with baseline (Figure 1). Anti-EBNA antibodies further decreased ($p < .01$) after flight (R+0 and R+3) as compared with baseline values. Anti-VCA was significantly increased ($p < .01$) at all three time points (L−10, R+0, and R+3) as compared with baseline. No significant difference was found in anti-measles antibodies at any time point.

The control group showed no significant change between baseline and day 15 samples in either the $\log_2$ VCA IgG antibody titers (9.2 ± 0.3 vs. 9.6 ± 0.2, $p = .2$) or the $\log_2$ EBNA IgG antibody titers (4.3 ± 0.4 vs. 4.4 ± 0.4, $p = .8$). In addition, no change was found in the antibody titers to the nonlatent measles virus (6.0 ± 0.5 baseline vs. 5.6 ± 0.4 at day 15, $p = .6$).

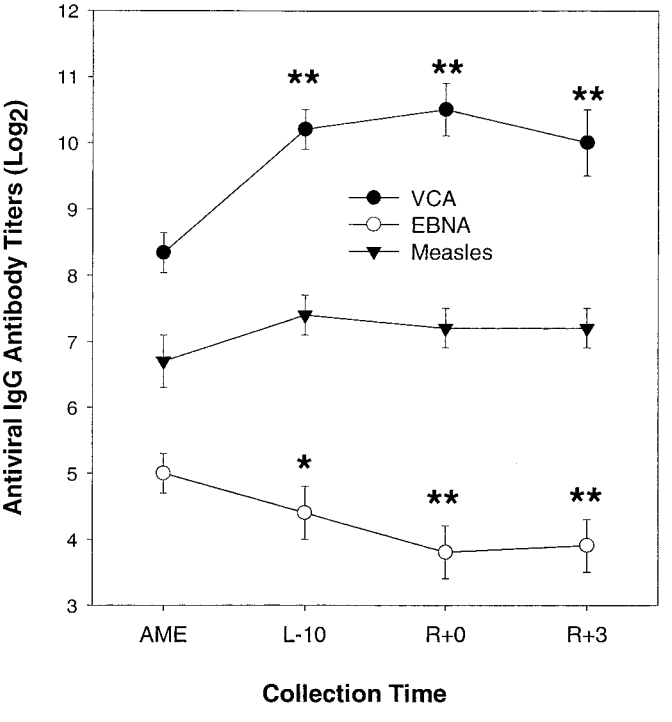

Fig. 1. Change in anti-VCA, anti-EBNA, and anti-measles virus IgG antibody titers before (AME and L−10) and after (R+0 and R+3) spaceflight. AME = annual medical exam (baseline). *$p < .05$; **$p < .01$.

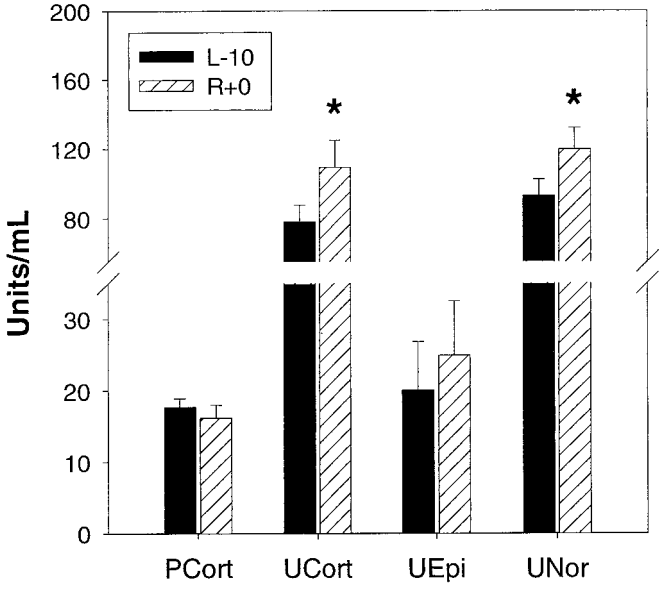

Fig. 2. Changes in plasma cortisol (PCort; μg/dl) and urinary cortisol (UCort), epinephrine (UEpi), and norepinephrine (UNor; all μg/24 h) at landing (R+0) as compared with preflight (L−10) levels. *$p < .05$.

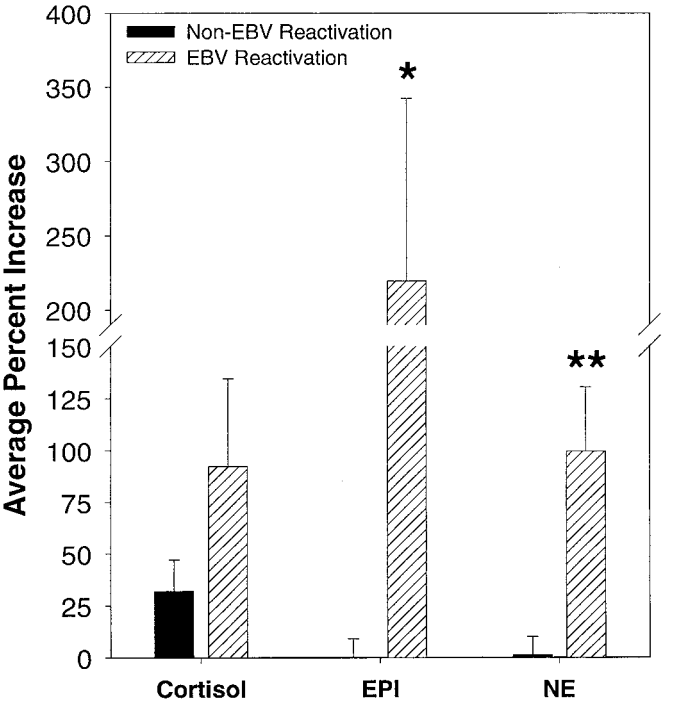

Fig. 3. Postflight changes in stress hormones in astronauts with or without evidence of EBV reactivation after spaceflight. Cortisol = urinary cortisol; EPI = urinary epinephrine; NE = urinary norepinephrine. *$p < .05$; **$p < .025$.

The postflight changes in neuroendocrine hormones are shown in Figure 2. No significant change was found for plasma cortisol ($p = .4$) or urinary epinephrine ($p = .058$). However, significant increases were found at landing for urinary cortisol ($p < .01$) and norepinephrine ($p < .05$). In our previous study (16), 11 of these subjects had serological evidence of EBV reactivation (defined as EBV-VCA/early antigen titers of 5120/160 or an eight-fold increase in IgG antibody titers). The stress hormone data were therefore grouped between those with and without evidence of EBV reactivation. No difference was found in the average percentage of change in plasma cortisol between the two groups (data not shown). No statistical difference ($p = .1$) was found in urinary cortisol for astronauts with EBV reactivation as compared with those without EBV reactivation (93% and 23%; Figure 3). Epinephrine and norepinephrine were increased in the EBV-reactivating group (220% and 100% vs. −1% and 1% in the nonreactivating group, respectively). These differences were statistically significant at $p < .05$ and $p < .025$, respectively. Interestingly, 9 of 11 astronauts in the EBV-reactivating group had a greater than 70% increase in epinephrine, as compared with 3 of 17 astronauts in the nonreactivating group ($p = .006$). In addition, the number of astronauts in the EBV-reactivating group with a 50% or more increase in norepinephrine was greater than the number in the nonreactivating group (10 of 11 vs. 2 of 17, respectively; $p = .001$).

TABLE 1. Subject Characteristics

	EBV Reactivation	No Reactivation
Age, y	42 ± 5	41 ± 5
Commanders	1	4
Pilots	3	2
Mission/payload specialists	6	12
Previous spaceflight experience		
None	5	11
1 flight	4	5
≥2 flights	2	1

Table 1 shows the characteristics of the astronauts with and without evidence of EBV reactivation. The ages for both groups were not significantly different. No difference was detected between the two groups when accounting for pilot/commander positions vs. mission/payload specialist positions ($p = 1.0$). Furthermore, no significant difference was found between first-time flyers and those with previous Shuttle mission experience ($p = .3$).

DISCUSSION

In the current study, further evidence was obtained consistent with stress-induced changes in immune

function before flight whereby anti-EBNA antibodies were decreased at L−10 as compared with baseline values. Moreover, anti-EBNA antibodies continued to decrease after spaceflight. These results are similar to those from psychological stress studies, in which examination stress resulted in decreased EBV-specific cytotoxic T-cell function and increased antibodies to latent herpesviruses (reviewed in Ref. 25). Although a limitation in this study was the lack of a baseline (ie, annual medical exam) sample for the control group, no change was observed in antibodies to the nonlatent measles virus for either group. Moreover, the increase in anti-VCA antibodies along with the lack of change in measles virus antibodies indicated reactivation of latent EBV in astronauts, and this occurred presumably as a result of stress. Indeed, the physical and mental rigors of training for a flight (ie, review of assigned flight tasks, public affair events, and family time) culminate in the few weeks just before the mission (communication from crew members). These results, along with previous findings (7, 16), support the hypothesis that cellular immunity is already decreased before spaceflight.

No increase in plasma cortisol was observed after spaceflight in the present study. One plausible explanation for this finding is variability due to the circadian rhythm of cortisol. The landing day blood samples were taken in the early afternoon, whereas the preflight samples were taken in the early morning. As such, it would be expected that plasma cortisol would be unchanged or decreased. However, a significant increase in cortisol was found as reflected by the 24-hour urinary values. This increase is thought to reflect an acute stress response to G-force acceleration encountered during atmospheric reentry (7, 18). In support of this notion, significant increases in plasma cortisol were found in blood samples taken with 30 minutes of 3-G centrifugation (26). Therefore, the lack of correlation between plasma and urinary cortisol values may reflect the difference in blood sampling vs. stimulus time (≈4 hours), and this interpretation is supported by the short half-life (~90 minutes) of circulating cortisol (27). Considering the multiple variables involved in spaceflight experiments (ie, G forces, time of postflight blood draws, length of missions, etc.), measurement of cortisol in urine samples is critical to accurately assess its release. Thus, 24-hour urinary cortisol levels may be a better overall indicator of stress than plasma levels.

Greater increases in catecholamines were found in the EBV-reactivating group as compared with the nonreactivating group. However, no significant differences were found in cortisol between the two groups. Glucocorticoids, but not catecholamines, have been shown to directly modulate reactivation of EBV (24), so EBV reactivation in these subjects cannot be attributed solely as a result of elevated cortisol. Interestingly, catecholamines have been shown to play an important role in modulating virus-specific $CD8^+$ T-cell function. Dobbs et al. (28) found that blockage of type II glucocorticoid receptors only partially restored virus-specific T-cell cytotoxicity in a murine model of herpes simplex infection; the addition of a β-adrenergic antagonist was required to fully restore this activity. Perhaps the increased catecholamine levels in the EBV-reactivating group significantly suppressed virus-specific T-cell activity in these individuals, resulting in enhanced viral reactivation and replication. Support for this hypothesis comes from the postflight decreases in anti-EBNA antibody titers, although limited plasma sample volumes resulted in antibody titer data for only 6 of 11 astronauts in the EBV-reactivating group, which precluded direct comparison of anti-EBNA values between the two groups. Thus, EBV reactivation in these astronauts may have resulted from both direct (ie, stress hormones) and indirect (ie, decreased immune function) mechanisms.

A common supposition is that first-time flyers and nonmilitary astronauts may be more susceptible to stress and other physiological changes associated with spaceflight. Buckey et al. (29) found that eight of nine presyncopal astronauts (89%) were either payload or mission specialists, and subsequent studies have found similar results when astronauts were grouped according to aviation experience (30). In contrast, we found no correlation of latent EBV reactivation between commanders or pilots and mission or payload specialists. In addition, no correlation was found between first-time flyers and those with previous spaceflight experience. These results indicate that EBV reactivation is not higher in any specific group or related to flight experience. However, it will be important to confirm these observations with a larger number of astronauts in future studies.

In summary, it will be important to determine the magnitude of "immune suppression" and herpesvirus reactivation in astronauts during spaceflight, which may be due in part to confinement, sleep deprivation, and acute changes in gravitational forces. In addition, immunological "recovery," which may vary with the time spent in space, will be important to investigate given the increased frequency of acute respiratory and other viral illnesses that have occurred after long-duration spaceflight (3). Although the clinical significance of these findings remains to be determined, recurrent herpesvirus infections (which are not mitigated by current measures limiting preflight access to astronauts) may be an important health concern on long-term spaceflights.

Our future studies will include direct measurement of virus-specific T-cells (ie, tetramers and ELISPOT) in conjunction with measurements of stress and latent herpesvirus reactivation to address these critical questions.

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